An Investigation of a Sono-Chemical Approach In Sterilization Problems

UNPUBLISHED PRELIMINARY DATA

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1. Purpose of Investigation:

The immediate objective of this investigation is to assess the feasibility of techniques designed to reduce the time presently required for the sterilization of vehicles employed in space exploration. The techniques under investigation include the use of acoustic vibrations and gases such as ethylene oxide. It is proposed that proper sonic and/or ultrasonic irradiations may enhance the antimicrobial properties of gaseous sterilizing agents.

2. Preparation of Spore Suspensions:

The resistant nature of bacterial spores makes them ideal test organisms for procedures intended to accomplish total sterilization. Two spore-forming bacterial species were selected for the present investigation: Bacillus subtilis var niger ATCC 9372 and Bacillus stearothermophilus ATCC 7953.

A variety of media were employed for the preparation of spore suspensions. Of the media studied, both liquid and solid, best results were consistently obtained with Nutrient Agar (Difco) supplemented with manganese (10 ppm) as recommended by Bruch, Koesterer, and Bruch (1963). Both B. subtilis var niger and B. stearothermophilus sporulate well in the latter medium. Spores of B. subtilis were washed 3 times with distilled water and pasteurized at 80 C for 20 minutes prior to use.

3. Preliminary experiments involving acoustic vibrations and ethylene oxide:

The spore suspensions of B. subtilis var niger, prepared as described above, were dispensed in 0.01 ml amounts on to filter paper strips measuring 1 X 0.25 inches. The inoculated strips were exposed to ethylene oxide, or acoustical vibrations, or both in a test chamber especially designed for this study. Following treatment, the test strips were suspended in sterile distilled water and homogenized in a blending apparatus. Plate

counts of the homogenates were made by subculturing to Trypticase Soy Agar (BBL).

Effect of ethylene oxide. Ethylene oxide was employed in a range of concentrations from 10 to 1000 mg/liter. The temperature was adjusted to 40 C and the relative humidity was set at 40%. Air pressure was reduced to one-half atmosphere. Complete kill occurred with ethylene oxide levels of 100 mg/liter or higher after an exposure period of one hour. Reduction of the exposure time to 30 minutes resulted in approximately 95% kill with lower levels of ethylene oxide.

Effect of sonic irradiation. Energy for both sonic and ultrasonic irradiations was supplied by a Multinsons Type 500-1 Generator (Macrosonics Corp.). The sound source employed here was a high power transducer (10 kc/sec) coupled to a specially designed velocity transformer for airborne emission. This transducer delivers 0.123 watt/cm^2 over an area of 7 cm^2 at the maximan distance allowed by the test chamber. Test strips were irradiated at 9.9 kcps at 40 C, 40% relation humidity, and at one-half atmosphere. Approximately 17% kill occurred after 4 hours irradiation. After eight hours, approximately 59% of the total population was killed.

Effect of ultrasonic irradiation. The sound source employed for these experiments was a high power transducer (23 kc/sec) coupled to a Friedmann-type velocity transformer. The latter transducer yields 0.35 watt/cm^2 over a 2.5 cm^2 area at the maximum distance obtainable in the test chamber. Test strips exposed to this sound source for 2 hours showed a reduction in the total population of approximately 14%. The percent kill increased to 41% after 8 hours irradiation.

Effect of ethylene oxide and sonic irradiation. Initial experiments were devised whereby test strips were exposed to ethylene oxide (10 mg/liter) and

sonic irradiation (9.9 kc/sec) simultaneously. The temperature was set at 40 C and a relative humidity of 40% was selected. Tests were conducted at one half atmosphere. Under these conditions, approximately 98% of the total population was killed. The latter figure was larger than those obtained for either ethylene oxide alone or sonic irradiation alone. Consequently, it appears that under specified conditions the use of ethylene oxide in combination with sonic irradiation may provide a greater bactericidal effect than the use of either alone.

4. Future Effort:

Experiments designed to ascertain the combined effects of ethylene oxide and acoustic vibrations will continue. Variations in the temperature, relative humidity as well as the sound waves selected will be employed. It is expected that additional transducers may be required. In addition, the effectiveness of positioning test strips closer to the sound source will be investigated.

5. Literature Cited:

Bruch, C. W., M. G. Koesterer, and M. K. Bruch. 1963. Dry-heat sterilization: its development and application to components of exobiological space probes. Develop. Ind. Microbiol. 4:334-342.